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AUTHOR(S):

Nakamura, Kaoru; Kondo, Hideyuki; Metzner, Patrick; Ohno, Atsuyoshi

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Lipase-catalyzed Asymmetric Transesterification of 3-Hydroxydithiopentanoate in an Organic Solvent

Kaoru NAKAMURA*, Hideyuki KONDO*[1], Patrick METZNER**
and Atsuyoshi OHNO*

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Chiral 3-hydroxydithiopentanoate has been prepared via resolution of racemic compound with a lipase in an organic solvent.

KEY WORDS: Asymmetric synthesis / Biocatalyst / Lipase / Enantioselective / Transesterification

The application of biocatalysts in selective transformations of synthetic substrates is a useful method to provide chiral building blocks of organic synthesis[2-5]. Especially, chiral 3-hydroxyalkanoates have been used as versatile chiral building blocks because of the facility to prepare these compounds asymmetrically by yeast reduction[6-8] or catalytic hydrogenolysis[9]. On the other hand, their sulfur analogs, chiral 3-hydroxydithioalkanoates have not received so much attention probably because of difficulty in preparing chiral derivatives. However, dithioalkanoates are known to have quite interesting properties and are receiving increased interest in aysmmetric syntheses as useful chirons[10, 11]. In the present paper, we would like to report kinetic resolution of ethyl 3-hydroxydithiopentanoate by using lipase-catalyzed transesterification in an organic solvent. The use of organic solvents in biocatalytic reactions has several advantages: 1) easy separation of biocatalyst from the product, 2) increase in solubility of nonpolar substrates, 3) protection of enzymes from inhibition by a substrate under high concentrations, 4) to keep the reaction system free from water-dependent side reactions.

Methyl 3-hydroxydithiopentanoate (**1**), which is not soluble to water and unstable under aerobic conditions, is a suitable substrate to exploit the reaction in organic solvents. We, therefore, investigated several lipases for their catalytic activity in transesterification of **1** with isopropenyl acetate as an acylating reagent as well as the solvent.

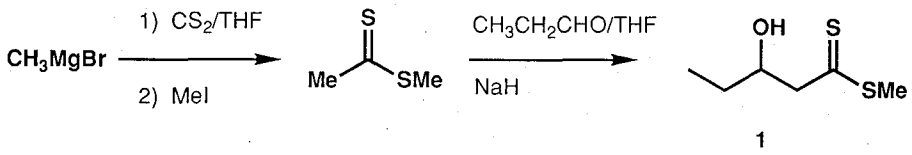
* 中村 薫・近藤秀行・大野惇吉: Laboratory of Bioorganic Chemistry, Institute for Chemical Research, Kyoto University, Uji, Kyoto 611, Japan.
** Patrick METZNER: Laboratoire de Chimie des Composés Thioorganiques, Université de Caen, 14032 Caen Cedex, France.

Results

Racemic **1** was prepared by the reaction shown in Scheme I; methyl dithioacetate was prepared by the reaction of methyl magnesium bromide with carbon disulfide, which was followed by methylation with methyl iodide[12]. The reaction of methyl dithioacetate with propionaldehyde in the presence of sodium hydride gave **1** in 9.9% overall yield. A lipase to be screened was added to a solution of **1** in isopropenyl acetate and the reaction was followed by HPLC. The results are listed in Table 1. Four lipases exerted the activities in transesterification and were used for further studies.

The enantioselectivity of lipase-catalyzed reaction was determined as follows. When the starting material was consumed about 50%, the reaction was quenched and the amount of the starting material unreacted as well as the enantiomeric excess (e.e.) in **1** and the corresponding acetate (**2**) were measured. The e.e. of the acetate was determined with ^{1}H NMR analysis using (+)-Eu(hfc)$_3$ as a chiral shift reagent. The e.e. of **1** was determined after converting it into the corresponding acetate chemically with AcCl/pyridine in benzene. The absolute configuration of the chiral **1** was determined by converting it into the corresponding carboxylic ester (**3**) as shown in Scheme 2. The results of lipase-catalyzed reactions are summarized in Table 2.

Scheme 1

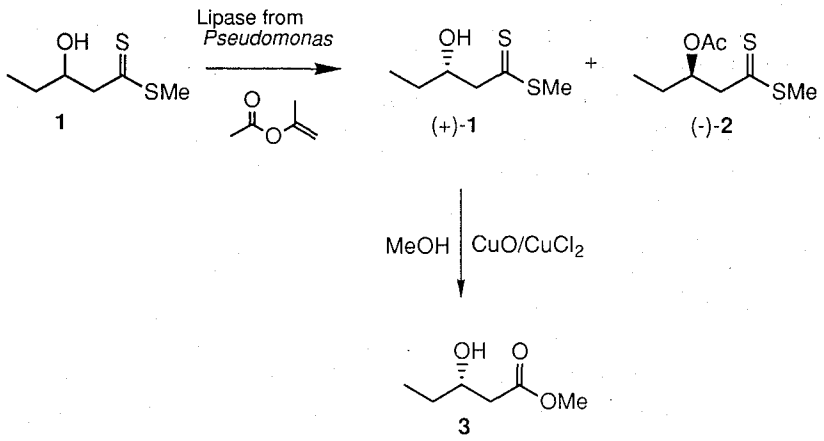


1

Table 1. Screening of lipases

Lipase (origin)	Activity
Amano AY (*Candida rugosa*)	+
Amano P (PCL) (*Pseudomonas cepacia*)	+
CCL (*Candida rugosa*)	+
Toyobo Type A (TA) (*Pseudomonas sp.*)	+
Amano A (*Aspergillus niger*)	−
Amano AK (*Pseudomonas sp.*)	−
Amano CE (*Humicola lanuginosa*)	−
Amano D-10 (*Rhizopus delamar*)	−
Amano F-AP15 (*Rhizopus javanicus*)	−
Amano G (*Penicillium sp.*)	−
Amano GC (*Geotrichum candidum*)	−
Amano L (*Candida lipolytica*)	−
Amano M (*Mucor javanicus*)	−
Amano N (*Rhizopus niveus*)	−
Amano R (*Penicillim rogueforti*)	−
PLE (*Porcine liver*)	−
PPL (*Porcine pancreas*)	−

Scheme 2



(-)-(*S*)-methyl 3-hydroxypentanoate

Table 2. Resolution of **1** with lipase

Lipase	Temp./℃	Time/h	Conv./%[a]	Yield/%[b]		$[\alpha]_D^{25}$		e. e./%(Config.)		E[14]
				1	2	1	2	1	2	
PCL	25	191	49	48	45	+18.9	−74.6	86(*S*)	95(*R*)	90
	40	60	46	44	43	+18.9	−62.0	86(*S*)	92(*R*)	67
TA	25	20	49	51	49	+14.5	−65.4	66(*S*)	71(*R*)	12
	40	6	54	42	44	+16.6	−55.7	77(*S*)	76(*R*)	12
AY	25	17	47	51	45	− 6.2	+28.7	25(*R*)	28(*S*)	2.2
	40	68	42	41	40	− 3.9	+26.7	12(*R*)	28(*S*)	2.0
CCL	25	400	43	45	44	− 5.2	+27.0	28(*R*)	30(*S*)	2.4
	40	315	45	44	37	− 4.2	+24.2	34(*R*)	21(*S*)	2.1

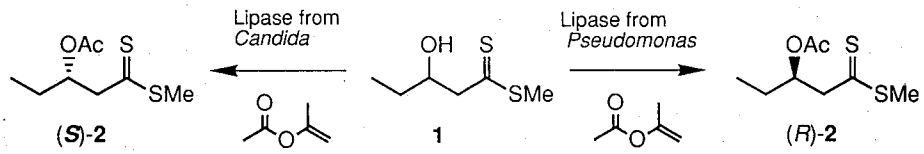
a) 100×[2]/([1]+[2]). b) Chemical yield, 100×[1 or 2]/([1] at 0 h).

Discussion

Four lipases exerted the activity in transesterification and, among those, Amano P (PCL) and Toyobo Type A (TA) are originated from *Pseudomonas*, and Amano AY (AY) and CCL are produced from *Candida rugosa*. Therefore, it is concluded that the lipases originated from those two species only can exert activity in transesterification. Furthermore, both lipases from *Pseudomonas* (PCL and TA) react with the (*R*)-enantiomer of **1** selectively and the other two lipases from *Candida* react preferentially with the (*S*)-enantiomer of **1** as shown in Scheme 3, although the selectivity of the latter enzymes are relatively low.

The lipase-catalyzed transesterification of ethyl 3-hydroxybutanoate (**4**), the oxygen version of **1**, in an organic solvent has been reported[13]. It is interesting to

Scheme 3



compare the reactivity and stereochemistry of **1** with those of **4**. The reaction of **4** proceeds in the order; CCL>PCL>PPL. However, PCL is much more active than CCL in the reaction with the dithioester, while PPL exerts no activity on it. Although PCL reacts with the (*R*)-enantiomer of both alcohols, CCL prefers the different enantiomer of **1** from that of **4**. It seems plausible that the cavity of the enzyme at the catalytic site is smaller in CCL than that in PCL and the bulky dithocarboxyl group is not accepted at the correct position of cavity in CCL. It should be noted that not only the change in stereochemistry but also tremendous retardation of the reaction is seen in the reaction of **1** with CCL.

Thus, the present reaction is worth to be employed for synthesizing chiral building blocks. Namely the reaction with PCL exerts a high enantioselectivity (E = 90)[14] in the transesterification, which indicates that resolution of **1** proceeds smoothly and selectively.

Experimental

Instruments

^{1}H NMR spectra were recorded on a Varian VXR-200 (200 MHz) in $CDCl_3$ with Me_4Si as an internal reference. Gas chromatography was recorded on a Shimazu GL 14 A gas chromatograph equipped with an OV 1701 capillary column (0.25 ㎜× 25 m) for analytical purpose, whereas for preparative separation, a Varian Aerograph Model 920 gas chromatograph was employed. HPLC was performed with a Hitach 655 Liquid Chromatography (pump) and IRICA 852-Ⅲ spectrometer, using a 4.6 × 150 ㎜ Wacosil 5 SIL column. Optical rotations were measured with a Perkin-Elmer 241 polarimeter.

Materials

Organic reagents were purchased from Nacalai Tesque Co. and Aldrich Chemical Co. unless otherwise indicated. Solvents and commercially available starting materials were generally used without additional purification unless otherwise indicated. Pyridine and benzene were refluxed on calcium hydride for 1 day and distilled before the use.

Preparation of Methyl 3-hydroxydithiopentanoate (1). Methyl dithioacetate[12].

A stirred mixture of methyl magnesium bromide (prepared from 0.6 mol of magnesium ribbon and 0.46 mol of methyl bromide) in 150 ml of tetrahydrofurane (THF) was cooled to − 10℃ and a THF solution of carbon disulfide (0.46 mol) was

added dropwise to the mixture. The resulted mixture was stirred at 60℃ for 1 h, then the mixture was cooled to 15℃ and a THF solution of methyl iodide (0.46 mol) was added dropwise. The whole mixture was stirred for 1 h. Ice-cold water (100 ml) and ether were added to the miuture successively and the organic materials were extracted. The etherial solution was washed with water and dried over anhydrous sodium sulfate. The solvent was removed *in vacuo* and the residue was distilled yielding 28.8 g (0.27 mol, 58.6%) of methyl dithioacetate: bp 44－45℃ / 22 mm Hg. ^{1}HNMR ($CDCl_3$) δ 2.61 (s, 3 H), 2.86 (s, 3 H).

Methyl 3-hydroxydithiopentanoate (1).

A THF solution of methyl dithioacetate (0.19 mol) and propionaldehyde (0.19 mol) was added gradually to a cooled (−20 ℃) mixture of sodium hydride (0.23 mol) in THF (30 ml). The reaction mixture was stirred for additional 30 min at the same temperature. Ice-cold water was added to the mixture and the mixture was acidified by diluted hydrochloric acid (pH 1). The organic materials were extracted with ether and the etherial solution was washed with water and dried over anhydrous sodium sulfate. The solvent was removed *in vacuo* and the residue was distilled yielding 6.4g (33 mmol, 17.3%) of methyl 3-hydroxydithiopentanoate (**1**): bp 85-93℃/5mm Hg. ^{1}H NMR ($CDCl_3$) δ 0.98 (t, 3H, J=7.4Hz), 1.50-1.65 (m, 2H), 2.64 (s, 3H), 2.87 (d, 1H, J=3.8Hz), 3.00-3.26 (m, 2H), 3.97-4.14 (m, 1H).

Recemic methyl 3-acetoxydithiopentanoate (rac-2).

Acetyl chloride (7.5 mmol) and pyridine (0.6 ml) were added successively to the solution of **1** (0.75 mmol) in dry benzene. After stirring for 1h, the reaction mixture was filtered and the benzene was removed from the filtrate *in vacuo* giving **2**. ^{1}H NMR ($CDCl_3$) δ 0.94 (t, 3H, J=7.4Hz), 1.60-1.75 (m, 2H), 2.03 (s, 3H), 2.62 (s, 3H), 3.15-3.40 (m, 2H), 5.28-5.42 (m, 1H).

Screening of lipases.

Isopropenyl acetate (1 ml) and **1** (0.36 mmol) were added to a lipase (60 mg) and the mixture was stirred under an argon atmosphere. At appreciate time intervals, 1 μl each of the reaction mixture was taken up and dissolved in 4 ml of dichloromethane and the solution was filtered through a short silica-gel column (Bond Elut). The filtrate was subjected to HPLC. Results are summarized in Table 1.

Transesterification with 1 under lipase-catalysis: general procedure.

Isopropenyl acetate (1 ml) and **1** (0.36 mmol) were added to a lipase (60 mg) and the mixture was stirred under an argon atmosphere. The reaction was monitored as described above. When the conversion reached about 50%, the reaction mixture was transfereed onto Bond Elute and was filtered by using dichloromethane (10 ml) as an eluent in order to remove the lipase. The fitrate was concentrated *in vacuo* and a part of the residue was subjected to ^{1}H NMR analysis in order to determine the amount of converted starting material. The sample used for the NMR anglysis was evaporated *in vacuo* and the combined residue was subjected to column

chromatography on silica gel using dichloromethane as an eluent yielding **1** and **2**, respectively. In order to determine the e. e. in the product, 17 mg of **2** and 34 mg (0.3 equivalent) of (+)-Eu(hfc)$_3$ were added to 0.6 ml of $CDCl_3$ and analyzed by ^1H NMR spectroscopy. The e. e. of **2** was determined by comparing the areas of signals from acetoxy-methyl protons in each enantiomer. Chemical shifts of products from the (*S*)-acetate appeared at higher field than those from the (*R*)-enantiomer. E. e. of **1** was determined after converting it into the corresponding acetate chemically with AcCl/pyridine.

Absolute configuration of chiral 1.

Absolute configuration of **1** was determined according to Scheme 2. Isopropenyl acetate (4 ml), **1** (1.4 mmol), and PCL (240 mg) were mixed and stirred under an argon atmosphere for 60 h. The reaction mixture was filtered through Bond Elut and washed with dichloromethane. The combined filtrate was subjected to a column chromatography as described above affording **1** (0.64 mmol, 46%, $[\alpha]_D^{25}$ $=+18.1°$ (c, 0.94 $CHCl_3$)) and **2** (0.60 mmol, 43%, $[\alpha]_D^{25}=-71.9°$ (c, 0.83 $CHCl_3$)). A solution of thus prepared (+)-**1** (0.59 mmol) in methanol (8 ml) was added dropwise to a mixture of copper(II) oxide (1.8 mmol) and copper(II) chloride (1.8 mmol) in methanol (8 ml). Water (0.1 ml) was added to the mixture and the resulted mixture was stirred for 12 h. The mixture was filtered and the filtrate was evaporated *in vacuo* and the residue was subjected to a preparative gas chromatography (PEG, 1.5 m, 120℃) yielding methyl 3-hydroxybutanoate (0.17 mmol, 22%, $[\alpha]_D^{25}=-29.4°$ (c, 0.87 $CHCl_3$)).

Since the optical rotation of (*R*)-methyl 3-hydroxydithiopentanoate was reported to be $-36.9°$, the absolute configuration of **1** obtained from the lipase reaction (unreacted starting material) was established as *S*.

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